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Abstracts

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Heparin and heparin analogs prevent the binding of immune complexes containing nucleosomal antigens to the GBM and delay nephritis in *MRL/I* mice. *M.C.J. van Bruggen, C. Kramers, M.N. Hylkema, R.J.T. Smeenk, G.W.K. van Dedem, and J.H.M. Berden, Department of Nephrology, University Hospital Nijmegen, Nijmegen, Department of Autoimmune Diseases, CLB, Amsterdam, and Diosynth BV, Oss, The Netherlands.* Recently, we found that monoclonal anti-nucleosome antibodies (mAbs) complexed to nucleosomal antigens can bind to DNA and to heparan sulfate (HS) *in vitro* and are able to bind to the GBM *in vivo* in a rat renal perfusion system, whereas non-complexed mAbs do not bind. Heparin was able to prevent the binding of these complexed mAbs to DNA and HS *in vitro* and to the rat GBM *in vivo*. When nucleosome/anti-nucleosome immune complexes are injected intravenously (i.v.) into *BALB/c* mice, GBM binding is observed. Pretreatment with heparin subcutaneously could prevent this binding. Also, heparin analogs devoid of anticoagulant activity could prevent binding of complexed anti-nucleosome antibodies *in vitro* and *in vivo*. We performed a therapeutic trial, testing the effect of heparin (H) and two heparin analogs (N-desulfated acetylated heparin (DSA-H) and low molecular weight N-desulfated acetylated heparin (LMW-H) on the development of albuminuria in *MRL/I* mice ($N = 15$ per group). Treatment was started at an age of 8 weeks and the dose given was 50 μg daily. In all three experimental groups albuminuria was significantly delayed ($P < 0.05$). In the table the percentage of mice developing albuminuria >1000 μg/18 hours is given.

	Age in weeks				
	12	14	16	18	20
PBS	0	6	13	40	60
H	0	0	13	13	13
DSA-H	0	0	7	7	14
LMW-H	0	0	0	0	6

Since delayed type hypersensitivity and the antibody response to a nominal antigen were not influenced by this treatment, we conclude that this effect was not due to immunosuppression. We conclude that interaction of heparin and heparin analogs with HS reactive immune complexes containing nucleosomal antigens prevents the binding of these immune complexes to the GBM and delays nephritis in *MRL/I* mice.

Heparan sulfate proteoglycans produced by glomerular visceral epithelial cells inhibit the proliferative effect of basic fibroblast growth factor on mesangial cells. *N.F. van Det, N.A.M. Verhagen, J.T. Tamsma, J. van der Born, J.H.M. Berden, J.A. Bruijn, M.R. Daha, and F.J. van der Woude, University Hospitals Nijmegen and Leiden, Departments of Endocrinology, Pathology, and Nephrology, Nijmegen and Leiden, The Netherlands.* Mesangial expansion is an important hallmark of diabetic nephropathy. It has been postulated that the decrease of glomerular heparan sulfate proteoglycan (HSPG) observed in diabetic nephropathy is important in this respect, since HSPG could inhibit mesangial proliferation and matrix production. Basic fibroblast growth factor (bFGF) may induce proliferation and extracellular matrix production in mesangial cells. It has been described that heparan sulfate is necessary for the receptor signaling of bFGF. We studied the interaction between glomerular HSPG and bFGF to see if the interaction with bFGF might explain the negative autoregulatory effects of HSPG. We cultured human adult mesangial cells until confluence and subsequently stimulated with 10 ng/ml recombinant bFGF for 24 hours. ^{3}H-thymidine (1 mCi/ml) was added for 6 hours and harvested. bFGF induced a 4–6 fold increase in proliferation compared to cells cultured under control conditions (DMEM + 0.5% Δ FCS). Glomerular HSPG isolated from human kidneys induced a significant inhibition of this mitogenic effect. Furthermore, we isolated HSPG from cell media and cell extract of cultured human adult glomerular visceral epithelial cells (GVEC) using two anion exchange columns and a superdex HR200 HPLC gelfiltration column. HSPG obtained from both cell media and cell extract of these cells inhibited the proliferative effect of bFGF on mesangial cells significantly. Heparitinase and nitrous acid treatment of these HSPG fractions reversed this inhibition. We conclude that heparan sulfate produced by GVEC inhibits the effect of bFGF on mesangial cells. Since heparan sulfate production by GVEC decreases under high glucose conditions, our findings suggest that a decrease in glomerular heparan sulfate is of importance for the observed mesangial expansion in diabetic nephropathy.

Anti-nucleosome antibodies complexed to nucleosomal antigens bind to cell surfaces and are internalized. Post-fixation migration of these antibodies leads to *in vivo* ANA. *C. Kramers, M.C.J. van Bruggen, G.P.M. Rijke, H.B.P.M. Dijkman, H. Croes, J. Fransen, R.J.T. Smeenk, and J.H.M. Berden, Departments of Nephrology, Pathology and Celbiology, University Hospital, Nijmegen, and Department of Autoimmune Diseases, CLB, Amsterdam, The Netherlands.* It has been suggested that binding of anti-DNA antibodies to cell surfaces, subsequent internalization, and nuclear binding (*in vivo* ANA) have pathophysiological consequences in SLE and may contribute to the development of nephritis. We have found previously that pathogenic anti-DNA antibodies can bind to heparan sulfate in renal basement membranes after complexing with nucleosomal antigens. Since it was found that nucleosomes can also mediate binding to cell surfaces and subsequent internalization, we hypothesized that *in vivo* ANA is a property of antibodies bound to nucleosomal antigens. We studied three anti-nucleosome mAbs which exhibit *in vivo* ANA in immunofluorescence (IF) when the hybridoma producing the mAb is inoculated intraperitoneally in mice. These antibodies complexed to nucleosomal material were injected intravenously (i.v.) in mice. After this injection in IF, *in vivo* ANA was observed, whereas injection of pure mAbs did not cause an *in vivo* ANA. In additional experiments with lymphoid cells we found that complexed antibodies bound to cell surfaces and were subsequently internalized in cytoplasmic vesicles, whereas pure non-complexed mAbs did not bind to cell surfaces and did not enter the cytoplasm. However, these complexed mAbs did not reach the nucleus in living cells. Because of this discrepancy, the *in vivo* experiments were repeated, a small part of the kidney was snap frozen in liquid N_2, fixed with acetone and studied in IF, whereas the rest was fixed *in vivo* by perfusion with PLP and studied in both IF and immunoelectron microscopy (IEM). After the former procedure ANA was observed; however, after PLP perfusion fixation nuclear binding was not observed. By IEM antibodies were identified in cytoplasmic lysosomes. In conclusion: anti-nucleosome antibodies complexed to nucleosomal antigens can bind to cell surfaces and are transported into the cytoplasm. Binding to nuclei is due to a post-fixation artifact. *In vivo* ANA found in patients might be due to this phenomenon. It is questionable,

therefore, whether this *in vivo* ANA has any pathophysiological significance.

Impaired endothelial function in patients with nephrotic range proteinuria (NS). *E.S.G. Stroes, J.A. Joles, P.C. Chang, H.A. Koomans, and T.J. Rabelink, Department of Nephrology, Academic Hospital Utrecht, Leiden, The Netherlands.* Proteinuria is associated with increased cardiovascular morbidity and mortality. Release of nitric oxide by the endothelium has been advanced as an important defense mechanism against vessel wall damage. In the present study we therefore tested the hypothesis that proteinuria is associated with endothelial dysfunction using venous occlusion plethysmography of the forearm. We infused L-NMMA (8 μmol/min), to inhibit basal nitric oxide (NO) production, serotonin (5-HT) (0.1, 0.3 and 1.0 ng/kg/min) to specifically stimulate NO release, and nitroprusside (SNP) (1, 10, 30 and 100 ng/kg/min) as an endothelium-independent vasodilator in the brachial artery of 9 NS (>3.5 g/24 hr) and normal renal function (creatinine 83.1 ± 8.7 μmol/liter). Control experiments were performed in 8 patients with active glomerulonephritis (GN) but normal renal function (creatinine 79.4 ± 5.4 μmol/liter) and 10 healthy volunteers (C). The intra-arterial infusions were repeated after local administration of excess L-arginine (1 μmol/kg/min), the substrate for NO synthase. Upon 5-HT infusion forearm vascular resistance (FVR) in NS decreased by 40% ± 5, which was significantly lower than in GN (56% ± 3; $P < 0.05$) and C (55% ± 3; $P < 0.05$). The calculated concentration, causing a 25% decrease in FVR (EC25; expressed as −log [molar]), was significantly higher in NS compared to GN and C (−8.26 ± 0.16 vs. −8.76 ± 0.08 and −8.81 ± 0.11; $P < 0.01$). The maximal decrease in FVR after SNP was not different between the groups (respectively 84% ± 2, 84% ± 3 and 84% ± 2; NS). The impaired 5-HT-induced vasodilation could be attributed to a defect in NO production, since L-NMMA almost completely prevented serotonergic vasodilation. In contrast, inhibition of basal nitric oxide activity by L-NMMA was not significantly different between these groups (FBF decreased by 30% ± 4, 39% ± 4 and 37% ± 2; $P = 0.15$). The defect in agonist-induced NO release could not be explained by decreased substrate availability, as infusion of excess L-arginine did not improve vasodilation. The nephrotic subjects also had increased lysophosphatidylcholine content in the low-density lipoprotein fraction, which has been shown to interfere with Gi protein-dependent signal transduction pathways, including 5-HT-induced vasodilation. In conclusion, nephrotic subjects demonstrate an impaired serotonin-induced nitric oxide release. This impaired vasodilatory response to serotonin, and thus decreased release and/or activity of nitric oxide, may imply a defective endothelial defense mechanism against vascular injury, and thus a major cardiovascular risk factor.

Videomicroscopic characterization of afferent and efferent arteriolar responses to angiotensin I and angiotensin II. *P.M. ter Wee, H. Forster, and M. Epstein, Nephrology Section, VAMC and University of Miami, and Department of Nephrology, Free University Hospital Amsterdam, Amsterdam, The Netherlands.* Utilizing a computer-assisted videomicroscopic technique, the effects of angiotensin I (Ang I) and Angiotensin II (Ang II) were investigated in isolated perfused hydronephrotic kidneys. In group 1, the long-term effect of Ang II (0.3 nM) on afferent (AA) and efferent (EA) arterioles was studied. In group 2, the microvascular effects of adding Ang I (0.3 nM and 1.0 nM) were assessed. In group 3, kidneys were pretreated with the ACE-inhibitor Trandolaprilat (TRAN; 10 μM), followed by the addition of Ang I in the same doses as group 2. At the end of this study, Ang II (0.3 nM) was added to assess the specificity of TRAN. In response to Ang II, AA ($N = 17$) manifested a persistent constriction (at 15 min −35% SE ± 2.3%; at 90 min −32.0% ± SE 1.8%). In contrast, EA ($N = 11$) manifested a biphasic response: an initial constriction of −38.5% ± 3.3% at 15 minutes was followed by a gradual dilation (at 90 min EA diameter averaged −16.5% ± 4.3% compared to baseline). Ang I induced an AA constriction ($N = 24$) of −14.7% ± 2.7% at 0.3 nM and −27.3% ± 2.4% at 1.0 nM. Ang I-induced EA constrictions ($N = 19$) were −10.2 ± 1.9% and −20.9 ± 2.4%, respectively. Pretreatment with TRAN attenuated the AA constriction ($N = 23$) at 0.3 nM Ang I (−6.5 ± 1.2%; $P < 0.025$ vs. Ang I alone) and at 1.0 nM Ang I (−12.7 ± 1.4%; $P < 0.001$ vs. Ang I alone). Subsequent addition of Ang II induced a constriction of −39.9 ± 1.8%. In EA ($N = 13$) pretreatment with TRAN completely prevented the Ang I-induced constriction (0.4 ± 1.3% at 0.3 nM Ang I, −1.2 ± 1.2% at 1.0 nM Ang I; both $P < 0.001$ vs. Ang I alone). After the subsequent addition of Ang II, EA constricted by −27.8 ± 3.3%. In conclusion, we demonstrated that AA respond to Ang II with a persistent vasoconstriction over time, whereas EA manifest a biphasic response. In addition, our observation that the ACE-inhibitor TRAN blunted the Ang I-induced AA constriction and prevented the Ang I-induced EA constriction, indicates that tissue-ACE participates in the local control of the renal microcirculation.

Increased transcapillary escape rate of albumin (TER_{alb}) and regional clearance of albumin (RC_{alb}) in long-term albuminuric diabetic rats. *J. van den Born, A.A. van Kraats, M.A.H. Bakker, and J.H.M. Berden, Department of Nephrology, University Hospital Nijmegen, Nijmegen, The Netherlands.* To investigate whether the development of albuminuria in experimental diabetes is a symptom of a more generalized vessel wall leakage, we measured the TER_{alb} and the RC_{alb} in several tissues one year after streptozotocin-induced diabetes in male Wistar-Münich rats. To this end 7 diabetic and 7 control rats were anesthetized with Inactin and received i.v. 2 μCi ^{125}I-albumin and 10 μCi ^{51}Cr-labeled red blood cells. The TER_{alb} was calculated from the disappearance rate of ^{125}I-albumin from the circulation, from 10–40 minutes after i.v. injection. Since ^{51}Cr red blood cells will not disappear from the circulation, this tracer enabled us to calculate the amount of extravascular albumin and RC_{alb} in the individual tissues. Results are shown in the table below; differences were tested in the unpaired Mann Whitney U-test.

Parameter	Diabetic rats	Control rats	*P* value
TER_{alb} *%/hr*	15.6 ± 2.6	13.3 ± 1.7	0.02
RC_{alb} *μl/g · min*			
kidney	2.04 ± 0.34	2.00 ± 0.15	NS
heart	1.91 ± 0.34	1.11 ± 0.13	0.0006
liver	1.36 ± 0.11	1.15 ± 0.12	0.007
lung	1.48 ± 0.23	1.95 ± 0.16	0.0012
skin	0.41 ± 0.07	0.40 ± 0.19	NS
muscle	0.11 ± 0.03	0.08 ± 0.02	0.04
aorta	2.26 ± 0.72	1.35 ± 0.24	0.04
Albuminuria *mg/24 hr*	31.7 ± 10.8	2.2 ± 1.5	0.0006
MAP *mm Hg*	118 ± 12	135 ± 6	0.003
Vascular volume/100 g body wt, *ml*	6.42 ± 0.29	4.65 ± 0.28	0.0006
Blood glucose *mmol/liter*	25.2 ± 2.2	5.0 ± 0.3	0.0006

These data clearly show that in diabetic rats an increased urinary albumin excretion is associated by increases in TER_{alb} and RC_{alb} in most, but not all, tissues. The fact that the TER_{alb} did not correlate with MAP, vascular volume or blood glucose, suggests that intrinsic capillary wall properties, rather than hemodynamic factors, are responsible for the increased vascular leakage of albumin in diabetic rats. Whether this is related to alterations of heparan sulfate in the vascular wall is under current investigation.

Intervention in experimental anti-MPO associated crescentic glomerulonephritis by monoclonal antibodies against adhesion molecules. *A.C. Muller Kobold, J.W. Cohen Tervaert, P.A. Klok, and C.G.M. Kallenberg, Department of Clinical Immunology and Pathology, University Hospital Groningen, Groningen, The Netherlands.* Renal failure frequently occurs in patients with anti-MPO associated systemic vasculitis. Histopathologically it is manifested as necrotizing crescentic glomerulonephritis without immune deposits. Recently, Brouwer et al developed a model of anti-MPO associated glomerulonephritis in the Brown Norway (BN) rat. To assess the possibilities of mitigating renal damage by blocking adhesion molecules, we evaluated the effects of mouse monoclonal antibodies to rat ICAM-1 (1A29) and rat CD18 (WT3) in this model. Distribution and half-life in the rat of 1A29 were determined by imaging techniques. For WT3, half-life was determined by Facs analysis. For both monoclonals half-life amounted to 24 hours. Next, BN rats were immunized with human MPO and subsequently disease was induced by perfusing the left kidney with products of activated granulocytes, that is lytic enzymes, MPO and its substrate H_2O_2. To study the effect of blocking adhesion molecules, rats were treated with 1A29 and WT3 or with 1A29 alone, whereas control rats were treated with an irrelevant monoclonal antibody (MOC31). Rats were injected every 2 days (1 mg/rat of each Moab), starting 2 days before disease induction. The experiment was terminated 5 days after disease induction. The following results were obtained: Urine, collected 24 hours

after disease induction, of rats treated with MOC31 or 1A29 alone contained red cell casts, while rats treated with both 1A29 ànd WT3 had normal urine sediments. At 5 days after disease induction, only a few lesions were observed in the kidneys of rats treated with the combination of 1A29 and WT3, while MOC31 treated rats had developed crescentic glomerulonephritis. No significant differences in cellular infiltrate could be found between 1A29 treated rats and MOC31 treated rats. In conclusion, blocking both ICAM-1 ànd CD18 appears to mitigate damage due to anti-MPO associated glomerulonephritis, while blocking ICAM-1 alone does not.

Deficient mucosal and systemic immune respons in patients with IgA nephropathy after repeated nasal immunization with cholera toxin subunit B. *J.W. de Fijter, J.W. Eijgenraam, C.A. Braam, J. Holmgren, A.W.L. van de Wall Bake, M.R. Daha, and L.A. van Es, Department of Nephrology, University Hospital Leiden, The Netherlands, and Department of Medical Microbiology and Immunology, Göteborg, Sweden.* Mesangial deposition of IgA is the hallmark of patients with primary IgA nephropathy (IgAN). The role of increased IgA plasma levels, circulating IgA-containing immune complexes and the mechanisms leading to mesangial deposition are still unclear. The abnormalities in the IgA immune system are restricted to the IgA1 subclass and there is accumulating evidence that the IgA1 in the mesangium may be bone marrow derived. The clinical association of upper respiratory tract infection with exacerbation of the disease and the results of previous immunization studies in IgAN lead to the hypothesis of a dysregulated "mucosa-bone marrow axis." Mucosal immunization with nonreplicating antigens generally induces both a local immune response at the site of immunization and a disseminated response at locations remote from the site of antigen encounter when mucosa associated lymphoid tissues are rechallenged. Infection with a replicating antigen also leads to a systemic immune response manifested by the presence of plasma antibodies. Cholera toxin subunit B (CTB) has been shown to induce not only a strong mucosal IgA response but also serum IgA (and IgG) antibodies. In this context we investigated the mucosal and systemic immune response in 12 patients with IgAN and 18 healthy volunteers after 3 subsequent intranasal immunizations with 0.33 mg of recombinant CTB (interval: two weeks). Simultaneous parenteral immunization with keyhole limpet hemocyanin (KLH) served as control. Mononuclear cells from peripheral blood (day: 0, 7, 21, 35, 42) and bone marrow aspirate (day 42) were isolated, and the IgM, IgG, IgA, IgA1 and IgA2 antibody secreting cells (antigen-specific and non-specific) were enumerated as spot forming cells (SFC/10E6) using the ELISPOT technique. Body fluids (plasma, nasal wash, saliva) were assessed for antigen-specific antibodies in ELISA. Statistical analysis was performed on logarithmically transformed data using a two-way analysis of variance with repeated measurements, followed by Scheffe's procedure for *post hoc* comparison. There was a significant immune response after immunization with both antigens in PBMC's and plasma for all isotypes tested ($P < 0.0001$). PBMC's from patients with IgAN showed a significantly lower number of CTB-specific IgA and IgA1 secreting cells after the first ($P < 0.005$, respectively $P < 0.0001$), the second ($P < 0.005$, respectively $P < 0.001$) and the third immunization ($P < 0.05$, respectively $P < 0.05$). The CTB-specific IgA and IgA1 plasma titers were also significantly lower in IgAN after the second and third dose of CTB ($P < 0.05$). There was no detectable specific mucosal immune respons in IgAN, reaching significance with controls after the third dose ($P < 0.0005$). The immune response after parenteral immunization with KLH was not different between patients and controls. At day 42 there was a significant correlation between the number of IgA and IgA1 SFC/10E6 cells ($r = 0.67$, $P < 0.005$, respectively $r = 0.76$, $P < 0.001$) and the number of CTB-specific IgA and IgA1 SFC/10E6 ($r = 0.68$, $P < 0.001$, respectively $r = 0.62$, $P < 0.001$) in the bone marrow with the respective concentration or titer of IgA or IgA1 in plasma. Two important conclusions can be made from the present study. First, our results support the existence of a mucosa-bone marrow axis in humans. Secondly, there is no systemic hyperresponsiveness in IgAN after mucosal immunization with CTB. In the population studied, patients with IgAN exhibit a deficient mucosal and systemic IgA1 immune response, despite the repeated challenge with a strong mucosal immunogen. No apparent dysregulation of this axis in IgAN was found in this study. In theory, this may implicate that patients with IgAN depend upon more frequent or prolonged antigenic encounter before adequate immunity at mucosal sites and systemic memory develops or that the type of antigen and its dependence on T cell help determine the ensuing IgA response.

Vasoactive effects of arginine vasopressin in healthy subjects and patients with a V2 receptor defect. *A.F. van Lieburg, V.V.A.M. Knoers, L.A.H. Monnens, T. Thien, and P. Smits, Department of Pediatrics, Human Genetics and Internal Medicine, University Hospital Nijmegen, Nijmegen, The Netherlands.* Studies assessing the vasoactive effects of arginine vasopressin (AVP) have yielded contrasting results, varying among species, between *in vitro* and *in vivo* studies, and among different vascular beds. Experiments in the human forearm vascular bed suggest that low dosages of AVP induce vasoconstriction, whereas higher dosages induce nitric oxide (NO)-mediated vasodilation. To elucidate the role of vasopressin type 1 (V1) and 2 (V2) receptors in this biphasic response, we investigated the vasoactive effects of AVP and 1-desamino-8-D-arginine vasopressin (DDAVP) in healthy volunteers as well as in patients with nephrogenic diabetes insipidus (NDI) with a proven V2 receptor gene defect. Venous occlusion plethysmography was used to assess the forearm blood flow (FBF) response to infusion of (DD)AVP into the brachial artery, both in the absence and presence of the NO-synthase inhibitor L NMMA (0.1 mg/min/dl). DDAVP (5–10–20 ng/min/dl) induced a dose-related increase of FBF from 2.3 ± 0.7 up to 10.3 ± 2.5 ml/min/dl in healthy subjects ($P < 0.01$, $N = 7$), but did not affect FBF in NDI (1.3 ± 0.3 to 1.3 ± 0.4 ml/min/dl, $N = 3$). In 2 healthy subjects, 7 incremental dosages of AVP (0.25 to 12.0 ng/min/dl) induced an initial decrease of FBF from 1.3 to 1.1 ml/min/dl, followed by a gradual increase to 3.1 ml/min/dl. The same dose range of AVP resulted in a gradual but progressive decrease of FBF from 1.2 to 0.4 ml/min/dl in NDI ($N = 1$). In healthy volunteers, infusion of L-NMMA reduced FBF from 2.3 ± 0.5 to 1.6 ± 0.2 ($N = 10$, $P = 0.05$). The increase in FBF induced by DDAVP (10 ng/min/dl) was significantly reduced by L-NMMA (5.7 ± 1.0 vs. 4.2 ± 0.8 ml/min/dl, $N = 10$, $P < 0.01$). We conclude that in humans, AVP-induced vasoconstriction is completely mediated by V1 receptor stimulation, whereas vasodilation induced by DDAVP or high dosages of AVP, is completely mediated by V2 receptor stimulation. This V2 receptor mediated vasodilation is at least partially mediated by release of NO.

The intracellular redox status modifies susceptibility to mercuric chloride-induced T cell activation. *J. Aten, N. Claessen, M.A. Chand, and J.J. Weening, Department of Pathology, University of Amsterdam, Amsterdam, The Netherlands.* In rodents, $HgCl_2$ can induce T cell activation leading in a genetically restricted way to either Th1-initiated immune suppression or Th2-dependent systemic autoimmunity. Since in an early stage of the syndrome $HgCl_2$-induced T cell activation is independent of MHC class II antigens, non-antigen-specific ways of cell activation seem to be involved in this process. The cellular response to $HgCl_2$ was examined using the murine T cell hybridoma 2B4.11 and human Jurkat and PBL T cells. $HgCl_2$ was found to cause Ca^{2+} influx as well as mobilization of Ca^{2+} from intracellular stores, to induce generation of free radicals, and to diminish intracellular glutathione (GSH) levels, the latter being accompanied by induction of apoptosis. Upon 24 hour incubation, the surviving cells displayed strongly increased GSH levels. $HgCl_2$-induced generation of oxidative stress was prevented by intracellular chelation of Ca^{2+}. $HgCl_2$-induced increase of both intracellular Ca^{2+} and free radicals was prevented by the reducing agent DTT; GSH, which is relatively cell membrane impermeable, was less effective. Neither DTT nor GSH prevented ionomycin-induced Ca^{2+} influx and oxidative stress. $HgCl_2$ stimulated the production of IL-4, but not of IL-2, by 2B4.11 cells. Inhibition of intracellular GSH synthesis enhanced the susceptibility to $HgCl_2$-induced cell death and increased $HgCl_2$-induced IL-4 production. Thus, $HgCl_2$ can induce cell activation leading to cytokine production and/or apoptosis. Susceptibility to both $HgCl_2$-induced cell activation and $HgCl_2$-induced cell death seems to be dependent on the intracellular redox status.

Differential changes in T-lymphocyte adhesion molecule expression during induction of immune dysregulation by mercuric chloride. *A. Roos, N. Claessen, J.J. Weening, and J. Aten, Department of Pathology, University of Amsterdam, Amsterdam, The Netherlands.* Injection of $HgCl_2$ induces a $CD45RC^{lo}$ TH2-dependent autoimmune syndrome in Brown Norway (BN) rats, involving glomerulopathy and proteinuria, and a TH1-dependent generalized immune suppression in Lewis rats. The induction of $HgCl_2$-induced autoimmunity is associated with MHC class II-independent T-T-lymphocyte interactions, which may involve altered expression or function of adhesion molecules on $HgCl_2$-exposed T-lymphocytes. Therefore, expression of T-lymphocyte adhesion molecules was studied in

BN rats ($N = 7$) and Lewis rats ($N = 6$) during induction of immune dysregulation by $HgCl_2$. Tri-color flow cytometry was performed on day 4 on peripheral lymph node cells of rats treated with $HgCl_2$ or H_2O. Following injection of $HgCl_2$, numbers of ICAM-1$^+$ cells in CD4$^+$ small cells were increased by 57% and 21% in BN and Lewis rats, respectively. CD4$^+$ T-cells from $HgCl_2$-injected BN rats showed higher LFA1 expression (+ 44%), increased fractions of CD45RC^{lo} cells (+ 14%), and increased fractions of OX40$^+$ cells (+ 78%) within CD45RC^{lo} but not within CD45RC^{hi} cells. In addition, CD45RC^{lo} blast cells of these rats showed modified expression of VLA4 (+ 33%) and CD43 (− 21%). These changes were not seen in Lewis rats. Numbers of CD26$^-$ cells were increased in CD4$^+$CD45RC^{lo} blast cells of both $HgCl_2$-treated BN rats (+ 290%) and Lewis rats (+ 88%). In contrast to BN rats, $HgCl_2$-treated Lewis rats showed an increase in the fraction of CD45RC^{hi} cells in their CD4$^+$ blast cell population (+ 29%). However, these cells did not show any abnormality in adhesion molecule expression. In summary, our results indicate that $HgCl_2$-injection induces altered adhesion molecule expression predominantly in CD4$^+$CD45RC^{lo} cells of BN rats. In CD4$^+$ cells of Lewis rats we only detected alterations in expression of ICAM-1 and CD26, and these changes were much less pronounced than those observed in BN rats. The differential effects of $HgCl_2$ on adhesion molecule expression in BN and Lewis rats may play a role in the immune dysregulation syndromes induced by $HgCl_2$ in these rat strains.

Anti-Thy-1 monoclonal antibodies (MAb) induce apoptosis of cultured rat glomerular mesangial cells (GMC). *T. Sato, J.G.A. van Dixhoorn, W.E.M. Schroeijers, T.W.J. Huizinga, C.P.M. Reutelingsperger, L.A. van Es, and M.R. Daha, Department of Nephrology, University Hospital Leiden, Leiden, and Department of Biochemistry, University of Limburg, Limburg, The Netherlands.* Apoptosis is a counteracting regulatory mechanism against undesired cell proliferation. Anti-Thy-1 nephritis is a model of mesangial proliferative glomerulonephritis. It has been reported that histological changes in Thy-1 nephritis are associated with the occurrence of apoptotsis. In the present study, we have investigated whether anti-Thy-1 MAb (ER4) is able to induce apoptosis of rat GMC *in vitro*. Quiescent rat GMC were co-cultured with RPMI 1640 containing 0.5% FCS alone, medium containing IgG2a anti-Thy-1 MAb, its F(ab')2 fragments or irrelevant control MAb, and were assessed for apoptosis qualitatively and quantitatively. In morphological studies with Hoechst 33258 stain, distinct nuclear fragmentation was observed with IgG2a anti-Thy-1 MAb and its F(ab')2 fragments. The effect was time- and dose-dependent. Apoptosis of cultured rat GMC also was detected by the dislocation of negatively charged phospholipid, phosphatidylserine, from the inner to the outer leaflet of the membrane. This method is a sensitive method for the measurement of apoptosis. Anti-Thy-1 MAb and its F(ab')2 fragments induced apoptosis of rat GMC in a time- and dose-dependent fashion, while medium alone, irrelevant control MAb and its F(ab')2 fragments displayed no significant apoptosis of cultured rat GMC. Upto 29% and 34% of cultured rat GMC displayed apoptosis with 1 μg/ml of IgG2a anti-Thy-1 MAb or its F(ab')2 fragments, respectively. Apoptosis was finally analyzed by gel electrophoresis. DNA extracts from cells co-cultured with IgG2a anti-Thy-1 MAb or its F(ab')2 fragments demonstrated typical internucleosomal DNA fragmentation. Taken together, the results demonstrate for the first time that IgG2a anti-Thy-1 MAb (ER4) is able to induce apoptosis of cultured rat GMC. The Thy-1 antigen on rat GMC therefore functions as one of the molecules regulating cell death and thereby determines the degree of mesangial alteration.

Infection of human renal mesangial cells with cytomegalovirus *in vitro* affects the production of complement components in a differential fashion. *J.J. Timmerman, M.F.C. Beersma, D.J. van Gijlswijk-Janssen, F.J. van der Woude, L.A. van Es, W.M. Spaan, and M.R. Daha, Department of Nephrology and Virology, University Hospital Leiden, Leiden, The Netherlands.* Human cytomegalovirus (HCMV) infection is widely spread in the human population. In individuals with deficient cell-mediated immunity (i.e. transplantation or AIDS patients) primary or secondary CMV infection leads to severe illness. *In vitro* infection of renal mesangial cells (MCs) with CMV results in an increased expression of MHC class I antigens on the cell surface of MCs, whereas MHC class II antigen expression is not affected. A number of complement components, namely factor B, C4 and C2, are encoded by the MHC class III region, positioned in between the MHC class I and II region. Previously, we showed that regulation of factor B synthesis by a number of defined cytokines, resembles MHC class I antigen expression, whereas the regulation of C4 synthesis resembles MHC class II antigen expression. In the present study, we investigated the effect of CMV infection of renal MCs on the production of the MHC class III encoded complement factors. First, a number of adult and fetal MCs were infected with CMV. While all adult MCs were susceptible to infection by CMV, fetal MCs were not infected by CMV. Next, we investigated the complement production of CMV-infected MCs. Factor B and C2 protein production was not detectable in control cells, but in the CMV infected cell populations an induction of both factor B and C2 occurred up to 250 pg/ml and 15 ng/ml, respectively. C4 synthesis was not detectable in both control and in infected cells. However, we were able to detect C4 synthesized by MCs when they were stimulated with IFN-γ. By Northern blot analysis we observed an induction of factor B mRNA in CMV-infected MCs, whereas the mRNAs for C2 and C4 were not detectable. RT-PCR analysis revealed induction of both factor B and C2 mRNA in the infected cell populations. Production levels of the regulator of the alternative pathway of complement, factor H, both in control and infected cells was 30 ng/ml. The steady state levels of factor H mRNA were not altered. CMV infection of the MCs resulted also in an up-regulation of IL-6 synthesis, both at the protein and mRNA level. In conclusion, we have shown that CMV infection of MCs resulted in an up-regulation of factor B and C2 synthesis both at the protein and mRNA level. Production of C4 and factor H was not affected by CMV infection. None of the fetal MCs were infected by CMV, indicating that they probably lack an (unknown) receptor for CMV on their cell surface.

Intrarenal injection of lipopolysaccharide in myeloperoxidase immunized brown Norway rats. *P. Heeringa, P.A. Klok, M.G. Huitema, P. Foucher, and C.G.M. Kallenberg, Departments of Clinical Immunology and Pathology, University Hospital Groningen, Groningen, The Netherlands.* Necrotizing crescentic glomerulonephritis (NCGN) associated with antibodies directed against myeloperoxidase (MPO) is characterized by fibrinoid necrosis of the glomerular capillary wall, marked infiltration of neutrophils and mononuclear cells, and paucity of immune deposits. Recently, Brouwer et al developed an animal model for pauci-immune anti-MPO associated NCGN in Brown Norway (BN) rats. This model is dependent on a MPO directed immune response and the presence of neutrophil lysosomal enzymes and H_2O_2 along the glomerular basement membrane. The present study adresses the question whether local activation of neutrophils in the presence of an anti-MPO directed immune response leads to the development of NCGN. For this purpose, BN rats were immunized with human MPO (10 μg/rat) in complete Freunds adjuvant (CFA) or with control solution in CFA. Sera were screened for the development of antibodies directed against human and rat MPO by ELISA and indirect immunoflourescence on ethanol fixed human and rat neutrophils. Two weeks after immunization a single injection of lipopolysaccharide (LPS, 1 mg/kg) in PBS was given in the left renal artery. Rats were sacrificed 1 and 7 days after injection and renal tissue was processed for histopathology including immunofluorescence and immunocytochemistry. BN rats immunized with human MPO developed a humoral response against human MPO and, to a lesser extent, to rat MPO. One day after LPS injection the number of intraglomerular neutrophils and monocytes was higher in the MPO immunized rats compared to control immunized rats. At day one, no intraglomerular immunoglobulin depositions could be detected in both groups. Seven days after LPS injection the number of intraglomerular neutrophils decreased in both groups, although still more neutrophils were found in MPO immunized rats. At this time point more intraglomerular monocytes were found in both groups compared to day 1 and more monocytes were found in MPO immunized rats compared to control immunized rats. However, inflammatory lesions characterisitic for anti-MPO associated NCGN had not developed.

Systemic and renal hemodynamic effects of tertatolol and atenolol in renal transplant recipients on cyclosporine A. *A.J.W. Branten, L.B. Hilbrands, H.W. van Hamersvelt, R.A.P. Koene, and F.Th.M. Huysmans, Department of Nephrology, University Hospital Nijmegen, Nijmegen, The Netherlands.* Cyclosporine A (CsA) contributes to hypertension after renal transplantation. Probably this effect of CsA is largely mediated by afferent renal vasoconstriction and is accompanied by decreases of RPF and GFR. In this situation, the beta blocker tertatolol (Ter) could be of special advantage since in hypertensive patients this drug has been proven to reduce BP with a simultaneous increase of RPF and GFR. However, little is known of the renal and antihypertensive effects of Ter and other beta

blockers in patients on CsA. Therefore, in 12 hypertensive renal transplant recipients (8 males, 4 females, mean age 45 ± 11 years) treated with CsA and a stable renal function 83 ± 61 weeks after transplantation, we studied the systemic and renal hemodynamic effects of Ter compared to atenolol (At) and to a period without any antihypertensive drug. Native kidney(s) were *in situ* in all. Patients were treated during periods of 4 weeks with At and Ter, respectively, separated by a wash-out period of 4 weeks. At the end of each period BP (Dinamap), heart rate (HR), GFR, and RPF (constant infusion of inulin and PAH, respectively) were measured. Results are given in the table.

	At	Wash-out	Ter
MAP, *mm Hg*	124 ± 2[a]	132 ± 4	125 ± 2[a]
HR, *b/min*	54 ± 3[a]	65 ± 3	55 ± 2[a]
GFR, *ml/min/1.73 m²*	45 ± 3	47 ± 4	45 ± 3
RPF, *ml/min/1.73 m²*	167 ± 15	163 ± 14	158 ± 11

Mean ± SEM; [a] $P < 0.05$ compared to wash-out, MAP = mean arterial pressure

Conclusion: Both At and Ter effectively reduced BP in patients on CsA. Since both drugs did not have any influence on GFR and RPF, Ter but also At can be safely used for the treatment of hypertension in renal transplant recipients on CsA. Our data do not explain the lack of a renal vasodilating effect of Ter in this specific group of patients.

Endogenous ACE inhibitors are present in rat cardiac and renal tissue but not in aorta and serum. *J. Koiter, G.J. Navis, P.E. de Jong, W.H. van Gilst, and D. de Zeeuw, GUIDE, University Hospital, Groningen, The Netherlands.* ACE inhibitor effects are reported to correlate with tissue ACE activity rather than serum ACE. Thus, accurate measurement of tissue ACE activity is of importance. However, pilot studies showed that sample dilution interferes with detection of ACE activity. Since different tissues require different sample dilution levels, both absolute activities and ratios between the ACE activities of different tissues could be incorrect. To establish this effect we measured ACE activity in serum, aorta, left ventricle and kidney at seven dilutions. Tissues and serum were obtained from male Wistar rats ($N = 6$). The ACE assay was performed with the substrate Hip-His-Leu. Released hippuric acid was measured by HPLC with UV detection. The initial sample dilution factor for serum, left ventricle, kidney, and aorta was 1, 5, 5, and 10, respectively. Serum was initially measured undiluted. ACE activity increased significantly with increasing sample dilution in homogenates of both left ventricle and kidney, but not in aorta and serum. The maximum left ventricle and kidney ACE activity (both 250% of the initial value) was reached when the samples were diluted 20 and 50 times, respectively. This *in vitro* dilution-induced increase in ACE activity suggests competitive inhibition of ACE by endogenous substances. This was confirmed by the fact that a known quantity of added purified ACE was inhibited by left ventricle and kidney homogenate: at 10% dilution, 49% and 36%, respectively. No inhibition of added ACE was observed in serum. In conclusion, the presence of endogenous ACE-inhibitors in cardiac and renal tissue strongly affects the measurement of ACE activity in tissue homogenates. For adequate assessment of the ACE activity in these tissues a series of sample dilutions is necessary. The (patho-) physiological role of these endogenous inhibitors *in vivo* remains to be established.

No effect of ranitidine on tubular creatinine secretion. *J.G. van den Berg, M.G. Koopman, and L. Arisz, Academic Medical Center, Amsterdam, The Netherlands.* Oral cimetidine competitively inhibits tubular creatinine secretion. We investigated the potential of oral ranitidine, another widely used H2-receptor antagonist, to block tubular secretion of creatinine, when given in regular (300 mg) to maximum (1200 mg) daily doses. In 10 normal subjects (5 males and 5 females, age 21–24 years), standard clearances of inulin and endogenous creatinine were simultaneously measured from 10 a.m. to 6 p.m. Inulin was administered by continuous infusion, starting at 8 a.m. After 2 hours equilibration, two 1.5 hour clearances were measured without ranitidine, followed by three clearance periods after ingestion of a single dose ranitidine at 1 p.m. All subjects participated in the first part of the study, when the effect of 300 mg ranitidine was studied; 4 subjects who showed the highest tubular creatinine secretion also participated in the second part, 3 weeks later, when 1,200 mg ranitidine were administered. As expected, neither dose of ranitidine caused a significant change in GFR, measured by inulin clearance and expressed as ml/min/1.73 m²: mean (±SD) baseline GFR was 128.7 (±20.3) versus 129.2 (±19.5) after 300 mg ranitidine, $P = 0.96$; in the 4 subjects who received 1,200 mg ranitidine 134.5 (±12.8), respectively 137.7 (±11.1) ml/min/1.73 m², $P = 0.72$. However, mean plasma creatinine did not change either: 65.9 (±8.4) μmol/liter before and 66.0 (±9.3) μmol/liter after 300 mg ranitidine, $P = 0.99$; 59.0 (±3.4) μmol/liter before and 58.5 (±2.0) μmol/liter after 1,200 mg ranitidine. Creatinine clearance (C_{Cr}) also remained unchanged [mean baseline C_{Cr} was 148.5 (±20.3) ml/min/1.73 m² vs. 149.1 (±20.3) after 300 mg ranitidine, $P = 0.95$ and 165.2 (±26.8) before vs. 158.2 (±16.0) after 1,200 mg ranitidine, $P = 0.67$]. Consequently, ranitidine had no effect on the mean creatinine relative to inulin clearance ratio [$C_{Cr}/C_I = 1.18$ (±0.10) before and 1.17 (±0.09) after 300 mg ranitidine, $P = 0.81$; baseline $C_{Cr}/C_I = 1.21$ (±0.13) before and 1.16 (±0.13) after 1,200 mg ranitidine, $P = 0.58$]. We conclude that a single oral dose of 300 to 1,200 mg ranitidine does not inhibit tubular secretion of creatinine. This lack of effect could be due to lower affinity of ranitidine for the transport carrier at the luminal tubulus membrane compared to cimetidine.

Cimetidine improves GFR estimation by the Cockcroft and Gault formula. *M.C.J. Ixkes, M.G. Koopman, B.A.C. van Acker, J.A. Weber, and L. Arisz, Academic Medical Center, Amsterdam, The Netherlands.* Creatinine clearance (C_{Cr}) overestimates true GFR because of tubular secretion of creatinine (TS_{Cr}). In patients with renal disease C_{Cr}/GFR can be as high as 2. Cimetidine inhibits TS_{Cr}, without influencing GFR, thereby reducing the overestimation of GFR. When standard C_{Cr} is measured, an important error may also be introduced by inaccurate collection of urine. To overcome this, C_{Cr} can be estimated from plasma creatinine, using the Cockcroft and Gault formula (C_{Cock}). We studied 19 patients with various renal diseases and plasma creatinine <180 μmol/liter (range 57–179). Proteinuria ranged from 0.02–8.4 g/24 hr (mean 2.96). We excluded patients with a Quetelet index (bw/l²) >30 or <15. During the 24 hours before GFR and creatinine determination the patients ingested orally 3 × 800 mg cimetidine. True GFR was measured as standard clearance using continuous infusion of I^{125}-lothalamate ($C_{I^{125}}$). Plasma creatinine concentrations were assayed by the enzymatic PAP reaction, creatinine in the urine by a modified Jaffé reaction. Plasma creatinine remained stable during the 6 hour period of GFR measurement, indicating that a new steady state was attained. The mean (±SEM) ratio of C_{Cock} to true GFR decreased from 1.28 (±0.049) before to 0.98 (±0.025) after administration of cimetidine ($P < 0.001$). Expressed as ml/min/1.73 m² the mean (±SD) difference from C_{Cock} to true GFR was 15.46 (±9.23) before and −2.51 (±7.07) after cimetidine. After cimetidine, the estimation of true GFR by C_{Cock} was as good as the estimation of GFR, obtained from two 2 hour creatinine clearances with urine collection ($P > 0.05$). Two patients had incomplete inhibition of TS_{Cr} ($C_{Cr}/C_{I^{125}}$ 1.21 and 1.12, respectively). These 2 patients had relatively low plasma cimetidine to creatinine ratios which suggests that the dose of cimetidine might have been insufficient. **Conclusion:** After cimetidine administration the Cockcroft and Gault formula more closely approaches true GFR, provided that the patient's Quetelet index is <30 >15. Therefore, it may be a useful method in the outpatient control of patients with renal disease and normal or moderately impaired renal function.

Steroid responsive human immunodeficiency virus associated nephropathy (HIVAN). *R.H. Kauffmann, R.B. Sie, J.A. Bruijn, Leyenburg Hospital, the Hague, and University Hospital Leiden, Leiden, The Netherlands.* A beneficial effect of prednisone in HIVAN has not been noted before. This report describes two patients with HIVAN, dysphagia and elevated liver enzyme levels who showed improvement with prednisone therapy. The first patient (a 34 year-old black male) was found to have renal insufficiency (serum creatinine 220 μmol/liter, creatinine clearance 40 ml/min), hypoproteinemia (protein 46 and albumin 21 g/liter), and proteinuria (4 g protein/24 hr urine) without urinary cells. AIDS was diagnosed 3 months previous due to a pneumocystis carinii pneumonia and HIV-1 seropositivity; maintenance therapy with cotrimoxazol was given. He complained of dysphagia and weight loss. Blood pressure was 135/80 mm Hg and edema was absent. The SGOT was 74, SGPT 46, LDH 567, GGT 246 and alkaline phosphatase 205 U/liter. His CD4 cell count was 30/mm³ and HIV p24 Ag was not detected. A liver biopsy showed steatosis and scanty infiltrates of mononuclear cells. The renal biopsy findings were compatible

with HIVAN showing focal segmental and global sclerosis, glomerular tuft collapse and swelling of glomerular and epithelial cells with hyaline drops on light microscopy and tubuloreticular structures in the endothelial cytoplasm on electron microscopy. Cotrimoxazol was discontinued and prednisone 60 mg/day was started. The serum creatinine fell to 120 μmol/liter, the albumin rose to 30 g/liter and the liver function tests became normal after 3 weeks of prednisone. After 8 weeks at a prednisone dose of 30 mg/day the creatinine was 90 μmol/liter. At that time he died of small bowel obstruction due to Kaposi sarcoma. The second patient (a 27 year-old black male, HIV-1 seropositive for 3 years) complained of dysphagia, 10 kg weight loss, and fever. He used cotrimoxazol 480 mg and nevaripine 400 mg/day. Blood pressure was 110/75 mm Hg and edema was absent. Serum creatinine was 250 μmol/liter, rising to 400 μmol/liter after 10 days, protein was 76 g/liter, and albumin 27 g/liter, decreasing to 21 g/liter. Liver enzyme levels were raised: SGOT 108, SGPT 102, LDH 394, GGT 730 and alkaline phosphatase 175 U/liter. His CD4 cells were 170/mm^3 and HIVp24 Ag was detectable. A liver biopsy showed moderate steatosis. The renal biopsy showed minimal glomerular sclerosis, swelling and proliferation of visceral epithelial cells with hyaline droplets and dilated tubuli containing proteinaceous casts on light microscopy and endothelial tubuloreticular cytoplasmic structures on electron microscopy, as in HIVAN. After treatment with prednisone, 60 mg/day for 3 weeks, the serum creatinine decreased to 170 μmol/liter, the albumin rose to 32 g/liter, and the liver function tests became normal except for the GGT. After 3 months the serum creatinine was 140 μmol/liter at a dose of 10 mg prednisone/day. Thereafter he became dependent on a dose of 20 mg prednisone to keep the creatinine level below 200 μmol/liter. These two cases of HIVAN accompanied by dysphagia and disturbed liver function show that steroid therapy may improve renal function in HIVAN.

Prognostic methods for mortality of intensive care (IC) patients with acute renal failure (ARF). *C.E. Douma, W.K. Redekop, J.H.P. van der Meulen, R.W. van Olden, J. Haeck, D.G. Struijk, and R.T. Krediet, Academic Medical Center, Amsterdam, The Netherlands.* Mortality of IC patients with ARF is between 60 and 80% and has not changed over the years. The aim of this retrospective study was to evaluate prognostic methods for mortality in these patients. Prognostic information may be useful for clinical decision making on the initiation of dialysis treatment. Of the 308 consecutive patients from 1985 to 1993 who were treated with dialysis for ARF in IC, 273 were included, and 35 patients (11%) were excluded because of incomplete data. Eight general prognostic methods and 4 methods, especially made for mortality prediction of patients with ARF, were used to calculate a score or mortality risk for each patient. The general methods were: APACHE II, Simplified Acute Physiology Score (SAPS), SAPS.Reduced (SAPS.R), SAPS.Extended (SAPS.E), Mortality Prediction Model (MPM), Sickness Scoring (SS), System Outcome Score (SOS), and Multiple Organ System Failure (MOSF). The ARF specific methods were described by the groups of Liano, Schaefer, Lohr and Rasmussen. After ranking for predicted mortality risk, patients were divided into 5 equally sized groups with increasing score or mortality risk (I–V). Predicted (PM) and observed mortality (OM) in these groups was calculated. Another way to compare methods is the Receiver Operating Characteristic (ROC) curve. The overall mortality was 78%. The PM of group I with the APACHE II was 14%, the OM was 76%, for group V the PM was 70% and the OM 87%. APACHE II underestimated the mortality. Furthermore, the difference in OM between patients with low (group I) or high (group V) predicted risk was very small. The predictive power of the MPM was somewhat higher: group I PM 4%, OM 61% and group V PM 66%, OM 89%. The SAPS.R PM in group I was 22% and OM 81%, in group V PM was 96% and OM 76%. SAPS.E: group I PM 18% and OM 63%, group V PM 73% and OM 77%. After calculation the score of Liano the OM in group I was 61% and in group V 94%. The remaining models showed mediocre performance. Only the method of Schaefer overestimated mortality: PM in group I was 88% and OM 63%, in group V PM 96% and OM 90%. The patient group of this study was most comparable with our patient group: IC patients with acute dialysis because of ARF. The areas under the ROC curves represent the predictive power of a method. They vary from 0.50 (SAPS.R) to 0.70 (MPM). In the part of the curve with the low false positive rate the contribution of all methods to prediction was low. **Conclusion:** General prognostic methods underestimate mortality of IC patients with ARF and the prognostic power of these methods is low. The prognostic methods especially made for patients with ARF give no better results. Therefore, none of these methods can be used for clinical decision making in IC patients with ARF.

The micturition of the conscious rat: Reliability of urine collection without catheterization. *M. Haas, C.A. Kluppel, D.K.F. Meijer, P.E. de Jong, F. Moolenaar, and D. de Zeeuw, GIDS, University Groningen, Groningen, The Netherlands.* Accurate urine sampling in conscious rats is often performed by invasive techniques with risk of infection. We tested the feasibility of timed urine collection, using a computerized sample collector in freely-moving spontaneously-voiding rats. The table shows that the frequency of urine voiding (U_{freq}) and the sample volume (U_{vol}) varied considerably over a 5 day period with a high individual variation coefficient (VC) per day. Since urinary creatinine excretion ($U_{cr}V$, HPLC measured) was far from its expected constancy over the day, the data suggest incomplete bladder voiding. Forced diuresis induced by infusion (0.45% NaCL/2.5% glucose 2 ml/hr) failed to reduce this biasing phenomenon. In both situations, with and without a forced diuresis, the large variation only allows detection of intervention-induced changes in diuresis and natriuresis of over 100%.

	Normal		
N = 5	Mean	CI	VC (%)
U_{vol}, *ml*	0.7	0–1.5	58
U_{freq}, *l/hr*	0.6	0–1.4	66
$U_{cr}V$, *μg/min*	14	0–28	52
UV, *μl/min*	7	0–14	48
UV, *μl/min*		4–10	22
$U_{Na}V$, *μEq/min*	2.0	0–4.2	56
$U_{Na}V$-c, *μEq/min*		4–16	30

	Forced diuresis		
N = 5	Mean	CI	VC *(%)*
U_{vol}, *ml*	1.5	0–3	50
U_{freq}, *l/hr*	1.1	0–2.5	64
$U_{cr}V$, *μg/min*	14	0–28	51
UV, *μl/min*	33	0–69	55
UV, *μl/min*		4–52	29
$U_{Na}V$, *μEq/min*	5.6	0–6.0	54
$U_{Na}V$-c, *μEq/min*		15–40	22

Interestingly, both the urinary flow (UV) and sodium excretion ($U_{Na}V$), closely correlated to the creatinine excretion (r = 0.85 and 0.70 without infusion, r = 0.88 and 0.82 with infusion for UV and $U_{Na}V$, respectively; all $P < 0.0005$). Thus, as shown in the table, creatinine correction of UV and $U_{Na}V$ (UV-c and $U_{Na}V$-c) resulted in a considerable reduction of the VC and 95% confidence interval (CI). Detection of 40% changes in both parameters becomes possible. We conclude that bladder voiding is often incomplete and/or the urgency to void is hardly related to the amount of urine present in the bladder. In spite of the increased voiding volume and frequency, constant infusion of NaCl/glucose does not result in a reduction of the degree of fluctuation. Correction of such incomplete bladder emptying is feasible using creatinine excretion data. It increases the sensitivity of detecting intervention-induced changes in urinary water and sodium excretion, and likely that of other urinary parameters.

Prevention of relapses of Wegener's granulomatosis by treatment with trimethoprim-sulfamethoxazole. A multicenter placebo-controlled trial. *C.A. Stegeman J.W. Cohen Tervaert, P.E. de Jong, and C.G.M. Kallenberg, Division of Clinical Immunology and Nephrology, University Hospital, Groningen, The Netherlands.* Wegener's granulomatosis is a frequently relapsing form of systemic vasculitis associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCA). Infections are suggested to trigger disease activity, a view supported by observations reporting beneficial effects of treatment with trimethoprim-sulfamethoxazole (co-trimoxazole). However, prospective controlled studies on the efficacy of co-trimoxazole as a therapeutic or prophylactic agent in Wegener's granulomatosis are lacking. We conducted a multi-center, prospective, randomized, placebo-controlled study with co-trimoxazole 800/160 mg b.i.d. during 24 months in 81 patients with Wegener's granulomatosis to

assess the efficacy of co-trimoxazole in preventing relapses. Relapses were scored using predefined criteria. The number of infections was assessed and serial determination of serum cANCA was performed. In 8 of 41 patients (19%) assigned to co-trimoxazole, treatment had to be stopped prematurely due to side effects. Seven of the 41 patients assigned to co-trimoxazole treatment relapsed during 24 months of follow-up as compared to 16 of 40 patients assigned to placebo [relative risk 0.40 (95% percent confidence interval, 0.17 to 0.98), logrank test]. A reduction in the number of respiratory ($P = 0.0046$) and non-respiratory infections ($P = 0.047$) was found with co-trimoxazole treatment as compared to placebo. No significant differences in cANCA titers between the groups were found. Proportional-hazards regression analysis identified treatment with co-trimoxazole [adjusted relative risk 0.32 (95% CI 0.13 to 0.79)] and a positive cANCA test at the start of the study [adjusted relative risk 2.89 (95% CI 1.12 to 7.45)] as independent risk factors for relapse. In conclusion, prolonged treatment with co-trimoxazole leads to a reduced incidence of relapses in patients with Wegener's granulomatosis in whom remission is induced with cyclophosphamide and prednisolone.

Improved ultrafiltration in CCPD patients using a glucose polymer (GP) instead of glucose (G) as last bag. *N. Posthuma, P.M. ter Wee, A.J.M. Donker, P.L. Oe, and H.A. Verbrugh, Department of Nephrology, Free University Hospital Amsterdam and Department of Clinical Microbiology, University Hospital Rotterdam, Rotterdam, The Netherlands.* Continuous peritoneal dialysis is associated with an increased risk of peritonitis and changes in the peritoneal membrane which can, in turn, lead to loss of ultrafiltration (UF) capacity. These disadvantages are long-term complications related to the use of hyperosmolar glucose solutions with a lack of biocompatibility. Recently, we started a randomized prospective study on the effect of glucose and glucose polymer (Dextrin 20; molecular weight 20,000 kD; 282 mOsm/kg) in automated peritoneal dialysis (APD) when used for the long daytime dwell. Of the 30 patients currently entered, 13 had a negative drain 0 at the start of the study. Because of this negative ultrafiltration during the daytime dwell, the administered volume had been reduced in several patients and some patients even had a "dry abdomen" during the daytime. This table showes the average UF (ml).

	Drain 0 (ml)		Total UF *ml/24 hours*	
Month	G(6)	GP(7)	G	GP
0	−435	−409	+754	+977
3	−347	+249	+837	+1311
6	−335	+233	+537	+1278

The six patients treated with glucose solutions manifested a persistently negative drain 0 after six months (Table). In contrast drain 0 became positive in 6 of 7 patients treated with the GP solution. In addition, in these patients the total UF improved. Thus, in all patients treated with the GP solution the CCPD treatment schedule could be adapted resulting in a reduction of the glucose concentration during the nightly exchanges or in the number of exchanges (as can be seen, because the drain 0-UF rose ±650 ml and the total UF 300–330 ml). As a result, the increase in allowed daily fluid intake together with the adapted treatment schedule improved the patients' subjective well-being, without complaints about the GP solution itself. In conclusion, the use of a Dextrin 20 solution enhances ultrafiltration, thus facilitating a less rigid restriction of daily fluid intake and/or treatment schedule resulting in an improved general well-being.

Balloon-expandable stent implantation in patients with renovascular hypertension due to atherosclerotic ostial renal artery stenosis. *P.J.G. vd Ven, J.J. Beutler, G.G. Geyskes, A.J. Woittiez, F.J.A. Beek, W.P.Th.M. Mali, and H.A. Koomans, Departments of Nephrology & Hypertension and Radiology, University Hospital Utrecht, Utrecht, The Netherlands.* PTRA of atherosclerotic ostial renal artery stenosis (RAS) is known for its high restenosis rate. We implanted a balloon-expandable stent (Palmaz) in 23 consecutive patients with renovascular hypertension due to atherosclerotic ostial RAS: 4 with unilateral stenosis, 7 with bilateral stenosis, and 12 with one-sided stenosis and an occluded contralateral renal artery. Mean BP was 190 ± 31/105 ± 11 mm Hg while on diverse medication. All patients had a positive captopril renography and/or a rise in serum creatinine during ACE-inhibition. Of the patients with bilateral RAS, 3 had bilateral ostial RAS and received bilateral stents. Thus, a total of 26 stents were placed. Angiographic results: Initial success rate was 96% (25/26 arteries). Follow-up angiography ($N = 13$, 15 arteries, mean 8 months, range 2–16) revealed significant restenosis in 13% (2/15). Clinical results: In the 12 patients with an occluded small contralateral kidney, ACE inhibition (ACEi) for BP control prior to stenting caused renal failure (median creatinine 249 μmol/liter, range 145–1,200). After unilateral stent placement (occluded kidney untouched) reasonable BP control (median BP 160/88 mm Hg, range 115–210/70–115) with ACEi became possible with better renal function (creatinine 152 μmol/liter, range 102–800). In the other 11 patients the median BP improved from 200/105 to 170/90 mm Hg, while plasma creatinine remained unchanged (median 126 μmol/liter, range 90–480, before, and 133 μmol/liter, range 75–605, after). Major complications occurred in 13% (3/23); renal artery dissection (1), cholesterol embolism causing decreased renal function (2), and toe amputation (1). In conclusion, these preliminary data show that atherosclerotic ostial RAS can be treated by balloon-expandable vascular stent implantation with satisfactory technical as well as clinical results: improvement of blood pressure control while renal function is preserved. In view of the severity of disease, the complications seem acceptable.

Evaluation of the Banff criteria for the histologic diagnosis of rejection in renal allograft biopsies. *Ph.M.M. Dooper, A.J. Hoitsma, R.A.P. Koene, and M.J.J.T. Bogman, Departments of Pathology and Medicine, University Hospital Nijmegen, Nijmegen, The Netherlands.* To assess the value of the recently developed Banff working classification of kidney transplant pathology for clinical practice, we compared retrospectively and blindly scored histological diagnoses, according to the Banff criteria, with the final clinical diagnoses in 210 renal allograft biopsies taken over a three year period in 145 patients with signs of graft dysfunction or proteinuria. The final clinical diagnoses were divided into five categories: acute rejection (AR), probable AR (prAR), equivocal (Eq), chronic rejection (CR), and no rejection (no R).

		Clinical diagnosis				
Banff diagnosis	*N*	AR	prAR	Eq	CR	no R
Borderline changes						
AR	38	8	6	0	8	16
Gr I	30	14	2	2	2	10
Gr II/III	118	85	13	7	5	8
CR/CsA/ATN/a.o.	24	1	1	0	9	13
Total	210	108	22	9	24	47

When the clinical diagnoses AR and probable AR are taken together and considered as clinical AR, the Banff criteria pointed to a correct diagnosis in 114 of the 130 rejection episodes, and in 46 of 80 cases without AR (that is, the total of cases with clinical diagnoses Eq, CR or no R). We conclude that the Banff classification system can serve as an acceptable guideline for a standardized histologic diagnosis of renal allograft rejection. A major shortcoming of the schema, however, is its overestimation of the diagnosis of acute rejection. Furthermore, 14 of the 38 biopsies with borderline changes correlated with clinical AR. Thus, the advice in the Banff classification that "borderline changes" are insufficient to justify antirejection treatment must be interpreted with caution.

Administration of OKT3 via continuous infusion attenuates first-dose side-effects. *S. Buysmann, C.E. Hack, F.N.J. van Diepen, J.M. Wilmink, S. Surachno, and R.J.M. ten Berge, Renal Transplant Unit and Clinical Immunology Unit, Department of Internal Medicine, Academic Medical Centre and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands.* The murine anti-CD3 monoclonal antibody OKT3 has proven its efficacy in prevention and treatment of allograft rejection. However, extensive use of OKT3 is limited by the clinical side effects which occur following the first dose administered as a *bolus* injection. These side effects have been attributed to a systemic release of cytokines, that is TNFα (Tumor Necrosis Factor α), IFNγ (Interferon γ), IL-2 (Interleukin-2), -3, and -6 and to immediate activation of complement and neutrophilic granulocytes. The latter is held mainly responsible for the early respiratory symptoms. Complement activation products themselves may induce release of cytokines, thus potentiating their noxious activities. Since complement activation occurs in a dose-dependent way, we investigated whether administration of

OKT3 as a *continuous infusion* during 2 hours could diminish the rapid complement activation and consequently the complement induced cytokine release and activation of neutrophils. In addition, we monitored whether a reduction of side effects occurred. Therefore, 18 renal allograft recipients were studied, who received 5 mg OKT3 prophylactically, either as a *continuous infusion* for 2 hours ($N = 9$), or as a single *intravenous bolus injection* ($N = 9$). Patients treated with *continuous infusion* experienced fewer side effects (score based on presence/absence of fever, chills, dyspnea, headache, diarrhea, nausea or vomiting, $P = 0.007$) and had a lower paracetamol consumption during the whole 10 day course. Peak levels of complement activation products C3a-desarg and C3b/c were significantly higher in the *bolus* group (22.34 ± 2.78, respectively 122.11 ± 16.05 nmol/liter) as compared to the *continuous infusion* group (9.82 ± 2.02 resp. 66.50 ± 18.86 nmol/l; $P = 0.007$ respectively, $P = 0.005$, Mann-Whitney test). In contrast no significant differences were observed between both groups in plasma cytokine levels (TNFα, IL-6, and IL-8) nor in activation of neutrophils as measured by plasma levels of elastase and lactoferrin. **Conclusion:** Administration of the first dose OKT3 as a *continuous infusion* for 2 hours induces fewer side effects as compared to *bolus* administration, the reduction of which is probably due to a lower degree of complement activation. Thus, in addition to cytokines, complement activation products seem to be pathogenic not only in the dyspnea occurring immediately after the first dose, but also in the side effects occurring later on.

Release of secretory phospholipase A_2 during OKT3 treatment and possible implications for renal function. *P.C. Wever, R.W. Roest, A.M. Wolbink-Kamp, G.J. Wolbink, J.J. Weening, C.E. Hack, and R.J.M. ten Berge, Academic Medical Center, Departments of Internal Medicine and Pathology, and Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.* OKT3, a murine monoclonal antibody of the IgG2a class directed against the CD3 molecule on T lymphocytes, is used in the prophylaxis and treatment of acute renal allograft rejection. Its use is followed by a transient nephrotoxic effect as reflected by an early rise in serum creatinine levels. An increase in circulating group II secretory phospholipase A_2 ($sPLA_2$) levels might account for this nephrotoxic effect, since $sPLA_2$ induces the biosynthesis of prostaglandins (PGs) and platelet-activating factor, which are known vasoactive lipid mediators influencing glomerular hemodynamics and renal function. We retrospectively studied plasma levels of $sPLA_2$ in 15 renal allograft recipients receiving OKT3 for the treatment of acute cellular rejection (ACR) and related these levels to circulating levels of tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6) and C-reactive protein (CRP) and to renal function. As a control group, we studied 15 renal allograft recipients receiving methylprednisolone (MPNS) for the treatment of ACR. In the OKT3 treated group $sPLA_2$ levels increased from 517 ± 223 ng/ml to peak levels of 2091 ± 693 ng/ml at 48 hours after the first drug administration (mean ± SEM, $P < 0.05$). The rise in $sPLA_2$ levels was preceded by significant increases in TNF-α and IL-6 levels and accompanied by a significant increase in CRP levels. Creatinine levels increased up to 127 ± 21% compared to pretreatment levels at 72 hours after initiation of treatment (mean ± SEM, $P < 0.05$), after which a gradual improvement in renal function was observed. In the MPNS treated group no increases in $sPLA_2$, TNF-α, IL-6, CRP and creatinine levels were found. Here, we show that administration of OKT3 results in cytokine-induced release of $sPLA_2$ and that peak levels of $sPLA_2$ are associated with increases in creatinine levels. Currently, we are prospectively studying the urinary excretion of PGs after OKT3 administration in relation to circulating $sPLA_2$ levels and renal function.

Acute renal allograft rejection is associated with increased excretion of monocyte chemoattractant protein-1. *W. Prodjosudjadi, M.R. Daha, J.S.J. Gerritsma, K.W. Florijn, J.N.M. Barendregt, J.A. Bruijn, F.J. van der Woude, and L.A. van Es, Department of Nephrology and Pathology, University Hospital Leiden, Leiden, The Netherlands.* There is a strong correlation between peritubular infiltration of monocytes and acute renal allograft rejection. It is not clear which factors regulate the influx of monocytes, but activation fragments of complement, adhesion molecules, and monocyte chemoattractant protein-1 (MCP-1) have been implicated. In the present study we have investigated the levels of MCP-1 in serum and the excretion of MCP-1 in urine of transplant patients with ($N = 9$) and without ($N = 11$) acute renal allograft rejection, represented by Rj and NRj groups respectively. MCP-1 staining of renal biopsies from the Rj group was performed. As controls, normal subjects ($N = 9$) and patients with ADPKD ($N = 14$) were included. Urinary excretion of MCP-1 as assessed by radioimmunoassay was significantly higher in the Rj group (938.7 ± 586.9 ng/mmol Cr) than in the NRj group (199.7 ± 122.4 ng/mmol Cr), the ADPKD group (118.9 ± 32.2 ng/mmol Cr), and the normal control group (53.5 ± 13.3 ng/mmol Cr). However, there was no significant difference in the levels of MCP-1 in serum between the various groups. The molecular weight of MCP-1 obtained from urine of patients with acute rejection was 13 and 11 kD, and both sizes were chemotactically active for monocytes. The intensity of MCP-1 staining on tubular epithelial cells from biopsies of renal allograft rejection was significantly increased as compared to the results from pretransplant biopsies. Seven out of 9 renal allograft biopsies showed an increase of more than 50% of total peritubular area, in the percentage of peritubular $CD14^+$ cells, as a marker for macrophages. In conclusion, we found that urinary excretion of MCP-1 is increased during acute renal transplant rejection. Serum levels of MCP-1 are not different between NRj, Rj, and normal controls. The observation that interstitial infiltration of macrophages is increased during a rejection process, combined with our previous findings that tubular epithelial cells produce MCP-1, suggest that local production of MCP-1 by tubular epithelial cells may be responsible, in part, for the recruitment of macrophages. In addition, urinary MCP-1 may therefore be derived from inflamed tubular epithelial cells or from infiltrating macrophages. Furthermore, in combination with other parameters, urinary excretion of MCP-1 seems to be a suitable marker for renal allograft rejection.